Total RNA/mRNA Isolation

Reagents: **RNA STAT-60: red solution (TEL-TEST "B", INC, Friendswood, TX)**
Choloroform, Isopropanol, Ethanol

Procedure:

1. Homogenization:

Attached cells: **Add 1ml/35mm dish RNA STAT-60 directly into culture dishes and pass the cell lysate several times through a pipette. (DONOT WASH THE CELLS)**

Non-adherent cells: **cells are sedimented then lysed in RNA STAT-60 (1ml/5-10 x 10^6 cells) by repetitively pipetting.**

a. **Add 0.2 ml of chloroform, cover the samples tightly, shake vigorously for 15 seconds, and then let samples stay at room temperature for 2-3 minutes.**
b. **Centrifuge samples at 12,000xg for 15minutes at 4^0C, and then transfer the aqueous phase (up clear lay) to a new tube.**

2. **RNA precipitation**
   a. **Add 0.5 ml of isopropanol into the new sample tube with aqueous phase, vortex to mix**
   b. **Store samples at room temperature for 5-10 minutes.**
   c. **Centrifuge at 12,000xg for 10minutes at 4^0C. RNA precipitate forms a white pellet at the bottom of the tubes**
   d. **Remove supernatant**

3. **RNA wash**
   a. **Add 1ml cold 75% ethanol into the sample tubes, vortex, and then centrifuge for at 12,000xg for 5 minutes at 4^0C.**
   b. **Pour the supernatant, dry the RNA pellet by lyophilizing for 3-5 minutes (DO NOT DRY COMPLETELY)**
   c. **Add 15ul DEPC water and pass the pellet through a pipette tip to dissolve the RNA pellet completely.**

4. **Measurement of yield**
   a. **Add 598ul of DEPC water into a new tube and add 2ul of RNA sample.**
   b. **Mix and measure OD at 260 nm (our machine reads: ug/ml)**